STUDIES ON THE PHYSIOLOGY OF THE EUGLENOID FLAGELLATES

III. The Effect of Hydrogen Ion Concentration on the Growth of Euglena gracilis Klebs[1]

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Introduction

Our knowledge of the effect of hydrogen ion concentration on the growth of the euglenoid flagellates is extremely scanty. Only a few organisms have been studied from this viewpoint, and in most cases the results are at best insufficient evidence for definite conclusions. Practically all the observations of this character have been limited to *Euglena gracilis*, except for those of Linsbauer (1915) and Turner (1917) on unidentified species and for the comparative studies of Kostir (1921), Mainx (1924, 1928), and Dusi (1930). The particular problem of the effect of hydrogen ion concentration on the growth of *Euglena gracilis* is one that has received considerable attention for several reasons. The organism is rather unique in that it possesses a very high resistance to acid solutions, and the literature on the subject is most confusing due to its contradictory character and to the fact that the results of most of the writers were obtained by neither accurate nor comparable methods. In most cases the actual hydrogen ion concentration was not determined, in some cases organic acids were used, in other instances the cultures were not bacteriologically pure, and in no case were quantitative methods employed. Therefore it was believed that an investigation, in which these factors of unknown importance were controlled, might prove useful in the development of culture methods and in the further study of the organism; for this reason the present study was undertaken.

This investigation was performed under the direction of Professor R. P. Hall, whom the writer wishes to thank for his advice during the course of the experiments and for his aid in the preparation of the manuscript.

[1] This paper, together with Parts I and II of this series of studies, was submitted to the Graduate School of New York University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, April 1, 1931.

Historical Survey

The unusually high resistance of *Euglena gracilis* to acid solutions was first recorded by Zumstein (1900), who used citric acid in his cultures in order to reduce the growth of bacteria. He found that *E. gracilis* grew well when 1–2 per cent citric acid was added to the 'earth infusion' used as a medium. Likewise, he obtained very good cultures with .5 per cent peptone to which he had added as much as 4 per cent citric acid. However, he obtained only poor cultures with .5–1.0 per cent tartaric acid and no growth at all with .2 per cent oxalic acid.

Ternetz (1912) repeated the experiments of Zumstein and found that citric acid was non-toxic to *E. gracilis* in peptone, beef-extract, and earth infusions, whereas it was quite toxic in synthetic inorganic media. Furthermore, she was able to detect no difference in toxicity between lactic, tartaric, malic, and citric acids when present in equimolar concentrations.

Pringsheim (1912) performed the same type of experiments but failed to corroborate the findings of Zumstein. He found that when .5 per cent citric acid was added to the peptone medium, *Euglena gracilis* grew very poorly, but he was able to obtain very good cultures with .12 per cent or less citric acid in the same type of medium. Therefore he concluded that the high acid resistance reported by Zumstein was erroneous, and citric acid was non-toxic only in very high dilution. Linsbauer (1915), working with an unidentified species which Mainx (1928) believed was *E. Klebsii*, found that citric acid was certainly toxic in concentrations as low as .07 per cent. Turner (1917), using an unidentified species of *Euglena* in bacterized cultures, found that an alkaline medium was favorable for growth of the organism.

Kostir (1921) made a study of the comparative resistance of seven euglenoids to various concentrations of citric acid. He found that *Euglena gracilis* was far more resistant than the other species used. The order in decreasing magnitude of the resistance of the species studied was: *E. gracilis*, *Phacus anacoelus*, *E. oxyuris*, *E. chrenbergii*, *E. geniculata* (?), *E. acus*, *E. deses*.

Tannreuther (1923) found that his most healthy cultures of *E. gracilis* were strongly alkaline and that the poorest cultures were either acid or very slightly alkaline. Since his cultures were not bacteria-free, however, these results might have been due to factors other than hydrogen ion concentration.

The next study of the effect of hydrogen ion concentration on *Euglena gracilis* was that of Mainx (1924, 1928), who used bacteria-free cultures in a medium composed of inorganic salts and .25 per cent beef-extract. He found that the organisms grew very well in this medium if

it were neutral. He also obtained good cultures when citric acid was added to a final concentration of 1/400 normal, and fair cultures in media containing 1/100 normal citric acid. Furthermore, he obtained very poor growth in cultures containing 1/500 normal NaOH, and no growth in cultures containing 1/100 normal NaOH.

Dusi (1930), using bacteria-free cultures of *Euglena gracilis* in a medium composed of inorganic salts and beef peptone, performed a more complete series of experiments. The possible effects of organic acids were eliminated by using only HCl and NaOH to bring the medium to the desired pH value. The medium was prepared at six different hydrogen ion concentrations, the most acid tubes having a pH value of 3.5–4.0 and the most alkaline ones a pH value of 8.5–9.0. He found that the maximum density of the cultures was approximately the same in media with pH values from 4.5 to 8.5, but that the maximum density was attained sooner in the alkaline cultures. He accredits this to a higher rate of division in these cultures. In a later paper Dusi (1930*a*) has reported similar experiments with five other species of Euglena, namely, *E. pisciformis*, *E. stellata*, *E. anabaena* var. *minor*, *E. deses*, *E. Klebsii*.

At the time the present study was undertaken the question of the effect of hydrogen ion concentration on *Euglena gracilis* was highly controversial. The methods and results of Dusi (1930) seem much more accurate than those of previous workers, but even his results were at best qualitative and by no means quantitative. Therefore it was deemed advisable to perform quantitative experiments in an effort to determine the relationship existing between hydrogen ion concentration and the growth rate of *Euglena gracilis*.

Material and Methods

The bacteria-free strain of *Euglena gracilis* used in this series of experiments was obtained from the cultures of the Pflanzenphysiologisches Institut of the German University at Prague through the courtesy of Professor E. G. Pringsheim. Fortunately, *Euglena gracilis* was much better adapted for experiments of this type than most of the other available species, because of its more rapid rate of growth in bacteria-free cultures under known conditions.

The organisms were cultured in 16 × 150 mm. Pyrex tubes plugged with cotton. The tubes were maintained at a temperature of 28.30 ± .05° C. by partial immersion in a water bath designed to accommodate a battery of six 100-watt light globes eighteen inches above the water level. The culture tubes were inclined on a wire rack at an angle of 45° in order that the plugs would not block the path of the light.

The medium adopted for the series of experiments was as follows:

KNO_3	.50 gram
KH_2PO_4	.50 gram
$MgSO_4$	.25 gram
NaCl	.10 gram
$FeCl_3$	.05 gram
Partially hydrolyzed casein	5.00 gram
Distilled water	1000.00 cc.

This medium was formulated and selected because the nature and the relative proportion of the constituents do not change considerably with titration or with autoclaving, such as is the case with media containing ammonium or bicarbonate compounds, which are unstable in alkaline solutions, or calcium sulphate and phosphate, which are only slightly soluble in neutral or alkaline solutions. Furthermore, the medium is well buffered against changes in hydrogen ion concentration within the range in which it was used. *Euglena gracilis* may live in such a medium at pH 6.7 for four weeks without producing a pH change definitely detectable with brom thymol blue. The medium was made up in large quantities and then subdivided and placed in 500 cc. flasks. The medium in each flask was brought to the desired pH value by the addition of normal NaOH or normal HCl. The flasks were then plugged and autoclaved. Equal amounts (always 10 cc.) of the medium were then measured directly into the test tubes by means of a Schelbach side-arm burette graduated to .1 cc. The tubes were plugged with cotton and autoclaved and were kept in a cool place until used.

Stock cultures for inoculation were grown in 250 cc. Erlenmeyer flasks in the above medium at a pH of 7.0. Transfers were made from rapidly dividing stock cultures of 10 to 14 days of age in which practically all the organisms were in the flagellated condition. Inoculations were made by means of sterile 12-inch Mohr measuring pipettes of 1 cc. capacity. The stock culture was shaken for five minutes before inoculations were begun and was then reshaken before each inoculation. The usual bacteriological method of aseptic transfer was used.

Measurements of hydrogen ion concentration were made with a La Motte comparator. The pH value was determined after inoculation for one sample tube of each set, and the pH values were determined for all other tubes at the end of the experiment. Readings were, in general, accurate to one-tenth of a pH unit, and the final values never varied more than this amount from the initial pH value except where otherwise stated (Series III*a* and IV*a*).

The ability of the organisms to grow at various hydrogen ion concentrations was measured by comparing the initial concentration of organisms with the concentration in each tube at the end of a definite

time. The same method described in Part I (Jahn, 1929) of this series of studies was used for counting the flagellates. In all cases the number of organisms was counted in at least fifty cubic millimeters of each sample, and three samples were counted from each tube. In all cases the concentrations of at least two and usually three tubes were averaged in order to determine the position of each point on the concentration-pH curve.

Experimental Results

Four series of experiments were performed, and each series will be described in detail.

Series I

This series was of a preliminary nature. The medium used was the same as that described above, with the exception that the partially hydrolyzed casein used was composed of one sample of Difco Tryptophane Broth. The stock solution was brought to pH 2.0 by the addition of normal HCl, and then each flask was brought to the desired pH value by the addition of normal NaOH. The pH values of the medium after autoclaving ranged from 3.6 to 8.9. After inoculation at the beginning of the experiment the range was only from pH 3.9 to 8.3, due to the neutralizing effect of the 1 cc. of a rich stock culture in the same kind of medium at pH 6.7 which was used as an inoculum in each case. Four tubes were inoculated at each pH value to be tested, and one tube at each pH value was chosen at random and tested colorimetrically to determine the initial pH after inoculation. Three extra tubes at pH 7.0 were inoculated so that they could be used to determine the initial concentration for the series. The average initial count for the three tubes was .9 thousand per cc., and this was considered to be the initial count for every tube of the series.

At the end of five days the concentration in one tube of each pH value was determined. The concentrations in every case were between 5.7 and 6.4 thousands per cc., and, considering the fact that only one tube of each set was counted, this variation is within the experimental error and can not be considered further. It was decided to count the other tubes at a later time when differences, if present, would be more pronounced. The second count was made on the twelfth day after inoculation. The results are shown in Fig. 1. The curve shows two maxima, one at pH 3.9 and one at pH 6.8, and two minima, one at pH 5.5 and one at the highest pH value used, 8.3.

This bimaximal curve was unexpected, and an explanation was not immediately evident. However, since a trypsin-like enzyme had been reported for *Euglena gracilis* (Mainx, 1928), and since the optimum pH for the digestion of casein may be between pH 6.0 and pH 7.0, it was

presumed that the higher growth rate in this range could be explained on the basis of more available necessary food material derived by more complete digestion of the casein. However, this point was not proven, and the high growth rate at pH 3.9 was yet unexplained. It was thought possible that acid hydrolysis of the casein decomposition products might have led to the presence of a higher concentration of available food material in this more acid range. With this in view, a series of amino-nitrogen determinations were performed on unused portions of the medium made up at the same time as that used in the experiments.

The formol titration method of Sorensen was used to determine the relative amounts of amino-nitrogen present in the samples. Four determinations were made for each flask of medium tested, and in all cases 7.0 ± .3 cc. of N/100 NaOH was necessary to restore the pink color to the solution. These results, of course, showed no significant differences in amino-nitrogen content of the media at different pH values. However, only a slight hydrolysis might have given rise to decomposition products of very high growth-accelerating power, and a slight hydrolysis could hardly be detected by the method used.

Series II

This series was started before the final results were obtained from Series I, and it is in some respects a repetition of the former. However, the results are quite different. The initial concentration of the organisms after inoculation was 1.8, and the range was from pH 3.9 to pH 7.9. The final concentrations were determined at the end of ten days. The results are shown in Fig. 1. It is seen that the maximal growth occurred in the most acid tubes. The minimum present at pH 5.5 in the previous series has apparently shifted to 6.5, and the more alkaline minimum of the previous series has failed to make its appearance. It was believed that the minimum present in the acid range in these two series was due to the lack of some particular decomposition product present in the more acid and in the neutral and alkaline ranges. Therefore, it was decided to provide the organisms with a more varied mixture of casein decomposition products, to make the initial concentrations very low, and to make the final counts before the organisms became numerous enough to exhaust any one type of food material. Such experiments are described as Series III and IV.

Series III

The method used in this series was the same as that employed in the two preceding ones. The medium was composed of the same inorganic compounds, but the partially hydrolyzed casein consisted of

material from three different samples of Difco Tryptophane Broth, one of which was lighter in color and much more readily soluble than the other two, and of two samples of Difco Tryptophane Broth which had been subjected to peptic and tryptic digestion. One of these had been digested by pepsin for two days and by trypsin for two days; the other had been digested by pepsin for two days and by trypsin for four days. These two mixtures and the three samples of Difco Tryptophane Broth

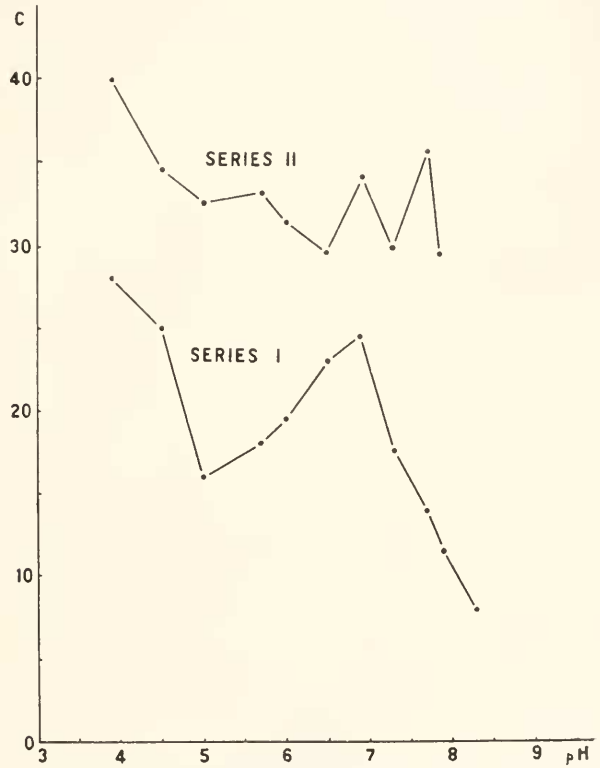

FIG. 1. Graph showing the results of Series I and II. The concentration of organisms in thousands per cc. (*C*) is plotted against pH. Each point represents the average of the concentrations of organisms in two or three tubes of the same pH value.

were mixed in approximately equal amounts. The stock solution of the medium was made up at pH 7.0, and then each sample was titrated to the desired pH value by the addition of normal HCl or normal NaOH.

The initial concentration after inoculation was .1 thousand per cc., and the pH range was 2.0 to 9.9. The concentrations of organisms were determined at the end of nine days. The data obtained are shown in Fig. 2. It is apparent that the organisms grew more rapidly

between pH 4.0 and pH 7.5 than in the more alkaline range. The optimum at pH 6.6 is still explainable as being due to the presence of a trypsin-like enzyme with an optimum at pH 6.7 or thereabouts. It is also evident that no growth took place between pH 2.0 and pH 3.6, and that little growth occurred at pH 9.9.

Series IV

This series was, in all essentials, a repetition of Series III. The initial concentration was .85, the pH range after inoculation was 2.0 to 9.9, and the concentrations of organisms was determined at the end of eight days. The results obtained are very similar to those of Series III, and they are also given in Fig. 2. The optimum at pH 6.6 will bear

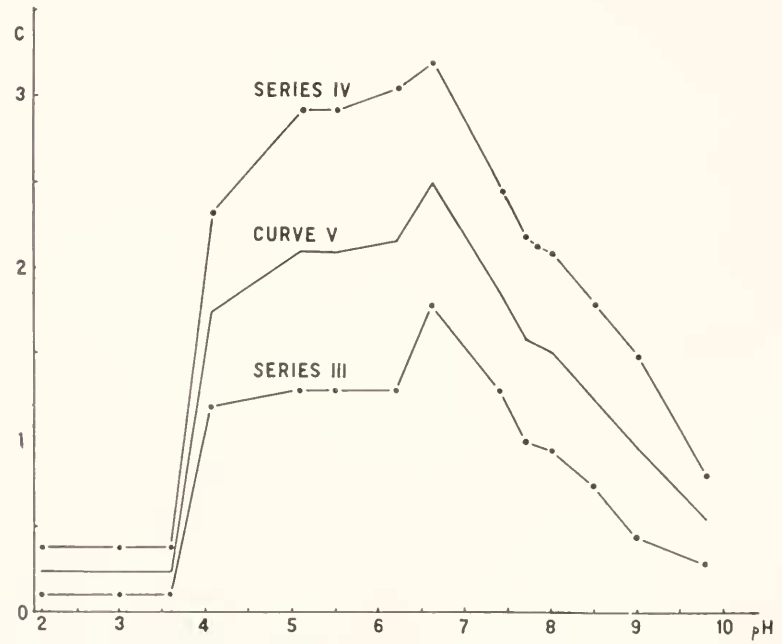

FIG. 2. Graph showing the results of Series III and IV. The concentration of organisms in thousands per cc. (*C*) is plotted against pH. Each point represents the average of the concentrations of organisms in two or three tubes at the same pH value. Curve V was obtained by averaging corresponding values of Series III and IV.

the same interpretation as that given above. Since the pH values of the tubes of Series IV correspond exactly to those of Series III, corresponding values were averaged, and the results were plotted as Curve V of Fig. 2. The curve shows a decidedly greater amount of growth between pH 4.1 and pH 7.5 than in the more alkaline range. The opti-

mum is at pH 6.6 and was probably due to the presence of more available necessary food material produced by the action of the trypsin-like enzyme.

Series IIIa and IVa

In each case the concentrations of the organisms in only two or three of the four tubes inoculated at each pH value in Series III and IV were determined in order to obtain the curves shown in Fig. 2. The other one or two tubes of each set were allowed to remain undisturbed and were examined at the end of seven weeks. At this time some of the organisms were encysted on the sides and bottoms of the tubes, and accurate counts were almost impossible. However, the results of macroscopic examination and of pH determinations are shown in Table I. Practically the same results were obtained in both

TABLE I

Initial pH	Amount of Growth	Encystment	Final pH
2.0	—	—	2.0
3.0	—	—	3.0
3.6	—	—	3.6
4.1	+	slight	4.2
5.2	++	slight	5.2
5.6	++	moderate	5.8
6.6	+++	moderate	6.8
7.5	+++	moderate	7.2
7.7	++++	very slight	7.4
8.0	+++	moderate	7.6
8.5	++	moderate	7.8
9.0	+	moderate	8.5
9.5	±	none	9.5

Key to the amount of growth:
— none.
± very slight.
+ slight.
++ moderate.
+++ abundant.
++++ very abundant.

series, and the two are summarized in the table. These results will henceforth be referred to as those of Series III*a* and IV*a* in order to distinguish them from the quantitative results obtained in Series III and IV at the end of 8–10 days.

Tests for a Proteolytic Enzyme

In order to confirm the existence of a proteolytic enzyme which might account for the optimal amount of growth at pH 6.6, inoculations were made into gelatin and into litmus milk media. Observations at

the end of four weeks showed doubtful liquefaction of gelatin and no appreciable effect on litmus milk. However, at the end of twelve weeks the gelatin cultures were almost completely liquefied, and considerable peptonization of milk and reduction of litmus were quite evident. These results confirmed the existence of a proteolytic enzyme as reported by Mainx (1928).

Discussion

At the time the present study was undertaken, the question of the relation of hydrogen ion concentration to the growth of *Euglena gracilis* was a highly controversial one. The results obtained by previous investigators were in a number of cases directly contradictory. The results of Zumstein (1900), Ternetz (1912), Pringsheim (1912), Kostir (1921), Tannreuther (1923), and Mainx (1924, 1928), although indicative of the effect of hydrogen ion concentration on *E. gracilis*, were complicated by at least one other factor such as the use of organic acids, inaccurate measurements of hydrogen ion concentration, or lack of bacteria-free cultures. The results of Dusi (1930) are not invalidated by such factors, but the observations were qualitative only and, as such, are not very informative as regards the effect of hydrogen ion concentration on division rate. The present investigation is an attempt to determine in a quantitative manner the relationship existing between the rate of multiplication of *Euglena gracilis* and the hydrogen ion concentration of the medium.

The curves presented in Fig. 2 may be taken as a measure of the ability of motile stages of *E. gracilis* to grow in a medium composed of certain inorganic salts and casein decomposition products at different pH values, and the curves of the two series of experiments seem to check as closely as might be expected. The maximum at pH 6.6 is probably due to the presence of a tryptic-like enzyme which exerts an optimum action on casein at pH 6.7. The presence of a proteolytic enzyme in cultures of *E. gracilis* has been demonstrated by Mainx (1928), and its existence is confirmed by the gelatin liquefaction and milk peptonization experiments of the author. The gradual decrease in the amount of growth with increasing alkalinity as shown in the curves from pH 6.6 to pH 9.9 checks very closely in both series and is quite the type of decrease that might be expected. The sharp rise from pH 3.6 to pH 4.1 might possibly be criticized if the range were not so great, but inasmuch as the range extended from pH 2.0 to pH 9.9, it was not practicable to use pH intervals smaller than those presented. However, such a sharp rise in growth-pH curves has been found in the case of acid-resistant bacteria. A similar sharp rise has been demon-

strated for *Escherichia coli* and *Bacterium aërogenes* (Cohen and Clark, 1919), and therefore it is not surprising that there should be such a phenomenon in highly acid-resistant protozoa such as *Euglena gracilis*.

The differences between the results of Series III and IV at the end of 8–10 days and at the end of seven weeks (Series III*a* and IV*a*) show that although the flagellates multiplied much more rapidly in the acid and neutral media for a short time after inoculation, the maximal density of population obtained after seven weeks was in tubes of pH 7.4–7.7. Inasmuch as Series III and III*a* were started at the same time from the same stock culture with the same initial inoculation and were maintained under the same conditions, and since the only difference between them is in the length of time the organisms were allowed to multiply, the shift in the optimum amount of growth from acid to alkaline media can not possibly be due to an experimental error. This is also true of Series IV and IV*a*, and the results of Series III and IV and of III*a* and IV*a* check very closely. This shift in the maximal amount of growth is very definite and very consistent in both pairs of experiments.

The results of the present investigation are not in direct contradiction to any of the results of previous workers. However, the fact that the division rate of *Euglena gracilis* is initially higher in acid cultures and that the maximum amount of growth is attained in the alkaline cultures is very useful in attempting to explain the contradictory and apparently valid results of previous investigators. The only disagreement between the present results and those of previous workers is with the results of Dusi (1930), who found that cultures of approximately the same density (macroscopic appearance) were obtained from pH 4.5 to pH 8.5 in a medium composed of beef peptone and inorganic salts. However, this might be due to differences in the time of observation in the two experiments, or perhaps to differences in the medium used.

The reason for such a shift in the maximal amount of growth with time is a matter of conjecture. One theory which may be presented is that there was some unknown limiting factor which inhibited growth in the acid cultures after the first few weeks. However, the possible nature of such a factor is totally unknown. Another theory which might be suggested is that the organisms inoculated into the acid solutions were temporarily stimulated to more rapid growth by the acid and that this stimulus failed to call forth a response after the first few divisions. However, the possible existence of such a growth-stimulating power of acid has not been demonstrated, and may not be disclosed by future investigation. Another theory is that certain hydrogen ion concentrations might induce temporary encystment with a concomi-

tant change in division rate. It has previously been observed that organisms transferred from a neutral medium to a strongly acid one may experience what has been termed an "inoculation shock" and may undergo encystment (Mainx, 1928). However, it seems likely that encystment would induce a temporary decrease in division rate, and therefore, this theory does not seem to be a likely explanation of the present phenomenon. If temporary encystment were accompanied by a temporary increase in division rate, the above results might be explained. Since practically nothing is known about the relationship which probably exists between encystment and hydrogen ion concentration and between encystment and division rate, and since encysted forms were not seen in appreciable numbers in Series III and IV, the importance of these factors in determining the above shift in maximal population can not be stated at this time.

The present results indicate that great care should be taken to determine the time relationships in experiments whose primary purpose is to determine the relationship existing between growth and hydrogen ion concentration. This is necessary in order that the early growth rate-pH relationships as shown in Series I, III, and IV are not overshadowed by other factors which become noticeably effective during a later period in the life of the culture, and which might give rise to later contradictory results such as shown in Series III*a* and IV*a*. It is not clear which of the two pairs of experiments represents the truer approximation to the usual growth rate-pH relationship existing in *Euglena gracilis*. The maximal growth in acid solutions as shown in Series III and IV might be explained as being due to a temporary growth stimulus exerted by the acid, and the maximal growth in alkaline solutions in Series III*a* and IV*a* as being due to limiting factors which prevented continued growth in the acid range. However, since it is somewhat unlikely that a growth-stimulating power of acid, if such exists, would show such a strong influence at the end of ten days, it seems more probable that Series III and IV are truer approximations of the usual growth rate-pH relationship.

Summary

1. The amount of growth of *Euglena gracilis* in cultures of different pH values has been measured quantitatively at the end of 8–10 days and has been estimated macroscopically at the end of seven weeks.

2. It is demonstrated that bacteria-free cultures of *Euglena gracilis*, in a solution of casein decomposition products and under conditions which allow mixotrophic nutrition, show, at the end of 8–10 days, a high growth rate between pH 3.9 and pH 7.5 with a maximum at about pH

6.6, and a uniformly decreasing growth rate with increasing alkalinity between pH 7.5 and 9.9.

3. It is also demonstrated that at the end of seven weeks the most growth is found to have occurred in the alkaline range, and that the maximal density of population is at about pH 7.5.

4. It is shown that the results of previous investigators, heretofore considered contradictory, may be explained on a basis of the time relationships involved.

5. The existence of a proteolytic enzyme in cultures of *E. gracilis* is confirmed.

LITERATURE CITED

Cohen, B., and W. M. Clark, 1919. The Growth of Certain Bacteria in Media of Different Hydrogen Ion Concentrations. *Jour. Bacteriol.*, **4**: 409.

Dusi, Hisatake, 1930. Les limites de la concentration en ions H pour la culture d'Euglena gracilis, Klebs. *Compt. rend. Soc. Biol.*, **103**: 1184.

Dusi, Hisatake, 1930*a*. Limites de la concentration en ions H pour la culture de quelques Euglènes. *Compt. rend. Soc. Biol.*, **104**: 734.

Jahn, Theo. L., 1929. Studies on the Physiology of the Euglenoid Flagellates. I. The relation of the density of population to the growth rate of Euglena. *Biol. Bull.*, **57**: 81.

Kostir, W. J., 1921. The Comparative Resistance of Different Species of Euglenidæ to Citric Acid. *Ohio Jour. Sci.*, **21**: 267.

Linsbauer, K., 1915. Notiz über die Säureempfindlichkeit der Euglenen. *Osterr. bot. Zeitschr.*, **65**: 12.

Mainx, Felix, 1924. Kultur und Physiologie einiger Euglena-Arten. (Vorl. Mitt.) *Lotos*, **72**: 239.

Mainx, Felix, 1928. Beiträge zur Morphologie und Physiologie der Eugleninen. I. Teil. Morphologische Beobachtungen, Methoden und Erfolge der Reinkultur. II. Teil. Untersuchungen über die Ernährungs- und Reizphysiologie. *Arch. f. Protist.*, **60**: 305.

Pringsheim, E. G., 1912. Kulturversuche mit chlorophyllführenden Mikroorganismen. II. Zur physiologie der Euglena gracilis. *Beitr. z. Biol. d. Pflanzen*, **12**: 1.

Tannreuther, G. W., 1923. Nutrition and Reproduction in Euglena. *Arch. f. Entw. d. Organism.*, **52**: 367.

Ternetz, Charlotte, 1912. Beiträge zur Morphologie und Physiologie der Euglena gracilis, Klebs. *Jahrb. f. wiss. Bot.*, **51**: 435.

Turner, C. L., 1917. A Culture Medium for Euglena with Notes on the Behavior of Euglena. *Anat. Rec.*, **12**: 407.

Zumstein, H., 1900. Zur Morphologie und Physiologie der Euglena gracilis, Klebs. *Jahrb. f. wiss. Bot.*, **34**: 149.